Title:

Biceps femoris architecture and strength in athletes with a prior ACL reconstruction

Authors:

Ryan G Timmins[1], Matthew N Bourne[2], Anthony J Shield[2], Morgan D Williams[3], Christian Lorenzen[1], David A Opar[1]

[1]School of Exercise Science, Australian Catholic University, Melbourne, Australia

[2]School of Exercise and Nutrition Sciences and Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

[3]School of Health, Sport and Professional Practice, University of South Wales, Pontypridd, Wales, UK

Corresponding author:

Ryan G. Timmins

School of Exercise Science, Australian Catholic University, 115 Victoria Parade, Fitzroy, 3065, Melbourne, Victoria, Australia

ryan.timmins@myacu.edu.au

Telephone: +61 3 9953 3742

Fax: +61 3 9953 3095

Running title:

Biceps femoris architecture and ACL injury

Disclosure of funding:

This study was partially funded by a Faculty of Health Research Grant from the Australian Catholic University.

ABSTRACT

Purpose: To determine if limbs with a history of anterior cruciate ligament (ACL) injury reconstructed from the semitendinosus (ST) display different biceps femoris long head (BF_{lh}) architecture and eccentric strength, assessed during the Nordic hamstring exercise, compared to the contralateral uninjured limb. **Methods:** The architectural characteristics of the BF_{lh} were assessed at rest and at 25% of a maximal voluntary isometric contraction (MVIC) in the control (n=52) and previous ACL injury group (n=15) using two-dimensional ultrasonography. Eccentric knee-flexor strength was assessed during the Nordic hamstring exercise. **Results:** Fascicle length was shorter (p=0.001; *d* range: 0.90 to 1.31) and pennation angle (p range: 0.001 to 0.006: *d* range: 0.87 to 0.93) was greater in the BF_{lh} of the ACL injured limb when compared to the contralateral uninjured limb at rest and during 25% of MVIC. Eccentric strength was significantly lower in the ACL injured limb than the contralateral uninjured limb (-13.7%; -42.9N; 95% CI = -78.7 to -7.2; p=0.021; *d*=0.51). Fascicle length, MVIC and eccentric strength were not different between the left and right limb in the control group. **Conclusions:** Limbs with a history of ACL injury reconstructed from the ST have shorter fascicles and greater pennation angles in the BF_{lh} compared to the contralateral uninjured side. Eccentric strength during the Nordic hamstring exercise of the ACL injured limb is significantly lower than the contralateral side. These findings have implications for ACL rehabilitation and hamstring injury prevention practices which should consider altered architectural characteristics.

Key Terms: Hamstring injury; eccentric strength; anterior cruciate ligament injury; fascicle length

INTRODUCTION

Paragraph 1

Anterior cruciate ligament (ACL) injuries are debilitating and result in a significant amount of time out from training and competition (5, 29, 30). In addition, a history of severe knee injury (including ACL injury) increases the risk of a future hamstring strain injury (HSI)(38). However, there has been little scientific investigation into why an athlete is at an increased risk of a HSI following an ACL injury (38). Reconstruction of the ACL following an injury is highly invasive and typically involves one of two types of autogenous grafts, harvested from either the semitendinosus/gracilis (ST) or patella tendon (8). These procedures, independent of graft type, have been reported to result in long term deficits in eccentric and concentric knee extensor(16, 17, 36) and flexor(19, 35, 36) strength up to 25 years following the reconstruction. Despite the known link between prior ACL injury and future HSI risk, research into compromised function of the knee flexors following ACL reconstruction, has mostly focused on strength (19, 36) and rate of force development(16). Investigations into structural differences of the hamstrings following ACL reconstruction have shown differences in hamstring muscle volume, with the gracilis and ST of the surgically repaired limb being significantly smaller, with the biceps femoris long head (BF_{lh}) being larger, when compared to the contralateral uninjured limb (33). However, the presence of other deficits in hamstring structure and/or function following ACL reconstruction remains largely unknown.

Paragraph 2

Of all the hamstring muscles, the BF_{lh} is the most commonly injured (18, 24). Therefore a greater understanding of the factors which might alter the risk of HSI in this muscle is needed. Recently it has been shown that limbs with a previous BF_{lh} strain injury display architectural differences when compared to the contralateral uninjured BF_{lh} (37). Most notably the previously injured BF_{lh} displays shorter fascicles compared to the contralateral uninjured muscle (37). It is well accepted that limbs with a previous hamstring strain injury display low levels of eccentric strength, which may be the result of (13, 27, 34) or cause (24) of injury. Since a previous ACL injury is considered a risk factor for a future HSI in athletes (18, 38) and considering the evidence which has shown reductions in eccentric strength in limbs with a previous ACL injury (19, 35, 36) , it is of interest to determine if alterations in hamstring architecture exist, given that eccentric contractions are thought to be a powerful stimulus for in-series sarcomereogenesis (3) and hypertrophy (31). As the BF_{lh} is the most commonly injured of the knee flexor muscles, it is also of interest to know if limbs with a previous ACL injury can lead, indirectly, to alterations in BF_{lh} architecture.

Paragraph 3

The purposes of this study were to: 1) determine if a limb with a previous ACL injury displays reduced eccentric knee flexor strength during the Nordic hamstring exercise when compared to the contralateral uninjured limb and a healthy control group and; 2) determine if the architectural characteristics of the BF_{lh} of the previous ACL injured limb is different to the contralateral limb without a prior history of ACL injury and a healthy control group. It was hypothesized that the previous ACL injured limb will exhibit reduced eccentric strength and will present with shorter BF_{lh} fascicles when compared to the contralateral uninjured limb.

METHODS

Participants

Paragraph 4

Sixty seven males (n=67) were recruited to participate in this case-control study. Fifty two (n=52) elite athletes (age 22.6 ± 4.6 years; height 1.77 ±0.05m; body mass 74.4 ±5.9kg) with no history of lower limb injury and in the past 12 months and no history at all of ACL injury were recruited as a control group. Fifteen elite (n=15) athletes with a unilateral ACL injury history (age 24.5 ±4.2 years; height 1.86 ±0.06m; body mass 84.2 ±8.1kg) were recruited to participate and form the ACL injured group. All athletes in both groups were currently competing at national or international level in soccer or Australian Football. Inclusion criteria for the ACL injured group were; (i) aged between 18 and 35 years, (ii) date of surgery between 2004 and 2013, (iii) ACL reconstruction autograft from the ipsilateral ST, (iv) no history of HSI in the past 12 months and (v) returned to pre injury levels of competition and training. All ACL injured athletes reported standard rehabilitation progression as directed by the physiotherapist of their respective clubs (21) and reported the use of some eccentric hamstring conditioning at the time of assessment (10). The ACL injured athletes (9 soccer players and 6 Australian Rules Football players) were recruited to assess the differences in the BF_{lh} architectural characteristics, maximum voluntary isometric contraction (MVIC) knee flexor strength and average peak force during the Nordic hamstring exercise of their ACL injured limb and the contralateral uninjured limb. All participants provided written informed consent prior to testing which was undertaken at the Australian Catholic University, Fitzroy, Victoria, Australia. Ethical approval for the study was granted by the Australian Catholic University Human Research Ethics Committee.

Experimental design

Paragraph 5

The test-retest reliability of real-time two-dimensional ultrasound derived measures of muscle thickness, pennation angle and estimates of BFlh fascicle length at rest and during different isometric contraction intensities has previously been investigated (37). Nordic hamstring exercise strength was assessed using a custom made device (25). All participants (ACL injured group and control group) had their BF_{lh} architectural characteristics, eccentric and MVIC knee flexor strength assessed during a single session. All ACL injured athletes were assessed during early pre-season in their chosen sport (Soccer: June to July 2014, Australian Rules Football: November to December 2014).

BF$_{lh}$ architecture assessment

Paragraph 6

Muscle thickness, pennation angle and estimates of BFlh fascicle length were determined from ultrasound images taken along the longitudinal axis of the muscle belly utilising a two dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm) (GE Healthcare Vivid-*i*, Wauwatosa, U.S.A). The scanning site was determined as the halfway point between the ischial tuberosity and the knee joint fold, along the line of the BF$_{lh}$. Once the scanning site was determined, the distance of the site from various anatomical landmarks were recorded to ensure reproducibility of the scanning site for future testing sessions. These landmarks included the ischial tuberosity, fibula head and the posterior knee joint fold at the mid-point between BF and ST tendon. All architectural assessments were performed with participants in a prone position and the hip in a neutral position following at least five minutes of inactivity. Assessments at rest were always performed first followed by the isometric contraction protocol. Assessment of BF$_{lh}$ architecture at rest was performed with the knee at 0º (fully extended). Assessment of BF$_{lh}$ architecture during isometric contractions was always performed with the knee at 0º of knee flexion and preceded by a MVIC in a custom made device (25). Participants were positioned prone on top of a padded board with both the hip and knee fully extended. The ankles were secured superior to the lateral malleolus by individual ankle braces which were secured atop custom made uniaxial load cells (Delphi Force Measurement, Gold Coast, Australia) fitted with wireless data acquisition capabilities (Mantracourt, Devon, UK). Participants were then instructed to contract maximally over a five second period, and the instantaneous peak force was used to determine the MVIC. The active architectural assessment was performed in the same device at 25% of MVIC with the participants shown the real-time visual feedback of the force produced to ensure that target contraction intensities were met.

Paragraph 7

To gather ultrasound images, the linear array ultrasound probe, with a layer of conductive gel was placed on the skin over the scanning site, aligned longitudinally and perpendicular to the posterior thigh. Care was taken to ensure minimal pressure was placed on the skin by the probe as this may influence the accuracy of the measures (15). Finally, the orientation of the probe was manipulated slightly by the sonographer if the superficial and intermediate aponeuroses were not parallel. Reliability of the sonographer when assessing the BF_{lh} architectural characteristics has been reported previously(37).

Paragraph 8

Once the images were collected, analysis was undertaken off-line (MicroDicom, Version 0.7.8, Bulgaria). For each image, six points were digitised as described by Blazevich and colleagues (1). Following the digitising process, muscle thickness was defined as the distance between the superficial and intermediate aponeuroses of BF_{lh}. A fascicle of interest was outlined and marked on the image (Fig. 1). The angle between this fascicle and the intermediate aponeurosis was measured and given as the pennation angle. The aponeurosis angle for both aponeuroses was determined as the angle between the line marked as the aponeurosis and an intersecting horizontal line across the captured image (1, 14). Fascicle length was estimated from the length of the outlined fascicle between aponeuroses. As the entire fascicle was not visible in the field of view of the probe its length was estimated via the following validated equation from Blazevich and colleagues (1, 14):

$$FL=\sin(AA+90°) \times MT/\sin(180°-(AA+180°-PA)).$$

Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation angle.

Paragraph 9

Fascicle length was reported in absolute terms (cm) and also relative to muscle thickness (fascicle length/muscle thickness). The same assessor (RGT) collected and analysed all scans and was blinded to participant identifiers during the analysis.

Eccentric hamstring strength

Paragraph 10

The assessment of eccentric hamstring strength using the Nordic hamstring exercise field testing device has been reported previously (25). Participants were positioned in a kneeling position over a padded board, with the ankles secured superior to the lateral malleolus by individual ankle braces which were secured atop custom made uniaxial load cells (Delphi Force Measurement, Gold Coast, Australia) fitted with wireless data acquisition capabilities (Mantracourt, Devon, UK). The ankle braces and load cells were secured to a pivot which allowed the force to always be measured through the long axis of the load cells. Following a warm up set, participants were asked to perform one set of three continuous maximal bilateral repetitions of the Nordic hamstring exercise. Participants were instructed to gradually lean forward at the slowest possible speed while maximally resisting this movement with both lower limbs while keeping the trunk and hips in a neutral position throughout, and the hands held across the chest. Following each attempt a visual analogue scale was given to assess the level of pain that was experienced. None of the participants reported any pain during testing. Verbal encouragement was given throughout the range of motion to ensure maximal effort. The peak force for each of the three repetitions was averaged for all statistical comparisons.

Data analysis

Paragraph 11

Whilst positioned in the custom made device, shank length (m) was determined as the distance from the lateral tibial condyle to the mid-point of the brace which was placed around the ankle. This measure of shank length was used to convert the force measurements (collected in N) to torque (Nm). Knee flexor eccentric and MVIC strength force data were transferred to a personal computer at 100Hz through a wireless USB base station (Mantracourt, Devon, UK). The peak force value during the MVIC and the three Nordic hamstring exercise repetitions for each of the limbs (left and right) was analysed using custom made software. Eccentric knee flexor strength, reported in absolute terms (N and Nm) and relative to body mass (N/kg and Nm/kg), was determined as the average of the peak forces from the 3 repetitions for each limb, resulting in a left and right limb measure (25). Knee flexor MVIC strength, reported in absolute terms (N and Nm) and relative to body mass (N/kg and Nm/kg), was determined as the peak force produced during a 5 second maximal effort for each limb.

Statistical analyses

Paragraph 12

All statistical analyses were performed using SPSS version 19.0.0.1 (IBM Corporation, Chicago, IL). Where appropriate, data were screened for normal distribution using the Shapiro-Wilk test and homoscedasticity of the data using Levene’s test. Reliability of the assessor (RGT) and processes used for the determination of the BFlh architectural characteristics has previously been reported(37).

Paragraph 13

At both contraction intensities, a split-plot design ANOVA, with the within-subject variable being limb (left/right or uninjured/ACL injured, depending on group) and the between-subject variable being group (control or ACL injured group) was used to compare BF_{lh} architecture, MVIC and Nordic hamstring exercise strength variables. For the control group, all architectural and strength measurements from the left and right limbs were averaged, as the limbs did not differ ($p>0.05$; Table 1.), in order to allow a single control group measure. Where significant limb x group interactions were detected, post hoc t-tests with Bonferroni adjustments to the alpha level were used to identify which comparisons differed.

Paragraph 14

Further between group analyses were undertaken to determine the extent of the between limb asymmetry in BF_{lh} architecture, MVIC and Nordic hamstring exercise strength, in the control and ACL injured groups. The control group between limb asymmetry was determined as the right limb minus the left and then converted to an absolute value (34, 37), whereas in the ACL injured group asymmetry was determined as the uninjured limb minus the ACL injured limb. Independent t-tests were used to assess differences in the extent of the between limb asymmetry in the control compared to the ACL injured group. Bonferroni corrections were employed to account for inflated type I error due to the multiple comparisons made for each dependent variable. Significance was set at a $p<0.05$ and where possible Cohen's *d* (4) was reported for the effect size of the comparisons, with the levels of effect being deemed small ($d = 0.20$), medium ($d = 0.50$) or large ($d = 0.80$) as recommended by Cohen (1988).

RESULTS

Power calculations

Paragraph 15

Power analysis was undertaken *a-priori* using G-Power(7). The analysis was based on the anticipated differences between the ACL injured limb and the contralateral uninjured limb in the ACL injured group. Estimates of effect size were based on previous research investigating differences between limbs in athletes with a unilateral HSI history(37). This previous study reported differences in BF_{lh} fascicle length, between the previously injured limb and the contralateral uninjured limb, to have an effect size of 1.34 when assessed at rest. Therefore an effect size of 0.8 was deemed reasonable as a starting point. Power was set at 80% with an alpha of 0.05 returning a calculated sample size of 15. As a cross-reference to confirm this sample size calculation, previous studies that have used similar designs have used samples from 13 to 15(27, 28, 34, 37).

Participants

Paragraph 16

The participants in the ACL injured group were 10.1±8.1kg heavier and 6.1±0.06cm taller compared to the control group ($p<0.05$). All athletes from the ACL injured group had suffered at least 1 ACL injury in the past 9 years (median time since surgery = 3.5years [range = 1 year to 9 years]).

BF_{lh} architectural comparisons

Paragraph 17

A significant limb-by-group interaction effect was found for fascicle length and fascicle length relative to muscle thickness at both contraction intensities ($p=0.004$). Post hoc analysis showed that fascicle length and fascicle length relative to muscle thickness were significantly shorter in the BF_{lh} of the ACL injured limb compared to the contralateral uninjured limb in the ACL injured group at both contraction intensities (p<0.05, *d* range = 0.87 to 1.31; Table 1; Fig 2.). A significant limb-by-group interaction effect was detected at both contraction intensities (p=0.003) for pennation angle. Post hoc analysis showed that pennation angle was greater in the injured limb compared to the contralateral uninjured limb in the ACL injured group at both contraction intensities (p<0.05, *d* range = 0.87 to 0.93; Table 1; Fig 2.). Comparisons of muscle thickness displayed no significant main effects (p>0.05, *d* range: 0.27 to 0.42; Table 1; Fig 2.), however when comparing the ACL injured limb to the contralateral uninjured limb, at rest, there was a small effect size (*d*=0.42; Table 1; Fig 2.) where the uninjured limb was thicker than the injured. No significant differences in any BF_{lh} architectural characteristics were found when comparing either limb in the ACL injured group to the average of both limbs in the control group (p>0.05, *d* range=0.11 to 0.21).

Paragraph 18

Comparing the extent of between-limb asymmetry in all the BF_{lh} architectural characteristics in the control group to the ACL injured group, the asymmetry in fascicle length, fascicle length relative to muscle thickness and pennation angle was greater in the ACL injured group (p<0.05, *d* range = 0.86 to 1.13; Supp Table; Fig 3.).

Knee flexor strength measures

Paragraph 19

A significant limb-by-group interaction effect was found for average peak force during the Nordic hamstring exercise (p=0.001). Post hoc analysis showed that the ACL injured limb (269.9N±81.4) was 13.7% weaker than the contralateral uninjured limb (312.9N±85.1) in the ACL injured group (between limb difference: 43.0N; 95% CI = 7.2 to 78.7; p=0.022; *d*=0.51; Table 2). Independent of whether it was relative to body weight or an absolute measure of force or torque, the ACL injured limb was weaker than the average of both limbs in the control group (p<0.05; *d* range = 0.58 to 0.74). There were no significant relative or absolute differences in force or torque between the uninjured limb in the ACL injured group and the average of both limbs in the control group (mean difference: 7.1N: 95% CI = -39.4 to 53.5; p=0.763; *d*=0.08).

Paragraph 20

Between-limb asymmetry during the Nordic hamstring exercise was greater in the ACL injured group (between group difference 36.0N; 95% CI = 12.2 to 59.7; p=0.003; *d*=0.71; Supp Table.).

Paragraph 21

Comparisons of knee flexor MVIC strength of the ACL injured limb to the contralateral uninjured limb and the average of both limbs in the control group displayed no significant differences (p>0.05, *d* range= 0.34 to 0.45).

Paragraph 22

Finally, no significant differences were found when comparing the extent of between limb asymmetry in knee flexor MVIC between the ACL injured group and control group (between group difference: -3.8N; 95% CI = -34.7 to 27.1; p=0.807, *d*=-0.07; Supp Table.).

DISCUSSION

Paragraph 23

The major findings were that elite athletes with a unilateral ACL injury, which was reconstructed with a graft from the ipsilateral ST, had shorter fascicles and greater pennation angles in the BF_{lh} of the previously ACL injured limb than the contralateral uninjured limb both at rest and during a 25% MVIC. Furthermore, between limb asymmetry of fascicle length and pennation angle was greater in the previous ACL injured group than the control group. Moreover, eccentric strength during the Nordic hamstring exercise was significantly lower in the previous ACL injured limb when compared to the contralateral uninjured limb, whereas comparisons of isometric knee flexor strength displayed a small difference between limbs as determined by effect size (d=0.31). Additionally the previous ACL injured group had a greater between limb asymmetry in eccentric knee flexor strength compared to the control group. To the authors' knowledge this is the first study that has investigated the BF_{lh} architectural differences in a limb with a previous ACL injury, reconstructed from the ipsilateral ST, in comparison to uninjured limbs (both from the contralateral limb and the control group). In addition, no prior work has examined the between limb differences in eccentric strength during the Nordic hamstring exercise in individuals with a history of unilateral ACL injury.

Paragraph 24

Observations of shorter muscle fascicles and greater pennation angles have been reported in previously strain injured BF_{lh} compared to the contralateral uninjured limb (37). However, no prior study had investigated the effect that a previous ACL injury has on hamstring muscle architecture. Athletes in the current study with a prior ACL injury, reconstructed from the ST, have somewhat comparable BF_{lh} fascicle lengths in their injured limb, at rest (10.13cm±1.39; Table 1) and 25% of MVIC (9.08cm±1.38; Table 1) compared to previously strain injured BF_{lh} (rest: 10.40cm±1.12; 25% of MVIC: 9.50cm±1.10) (37). Additionally, the extent of between limb asymmetry in BF_{lh} fascicle length in the athletes from the current study, when assessed at rest (13.7%; 1.61cm±0.31) and 25% of MVIC (12.9%; 1.35cm±0.25) is comparable to individuals with a unilateral history of BF_{lh} strain injury (rest: 12.9%; 1.54cm±0.12; 25% of MVIC: 10.9%; 1.17cm±0.10) (37). The similarities in BF_{lh} fascicle length and between limb asymmetry in individuals with two different injuries are of great interest as a history of both ACL injury and HSI increases the risk of future HSI (18, 38). However the maladaptations which influence the increase in HSI risk in individuals with a previous ACL injury are unknown. It has been hypothesized that possessing shorter muscle fascicles, with fewer in-series sarcomeres, may result in an increased susceptibility to eccentrically-induced muscle damage (2, 22). Therefore the shorter BF_{lh} fascicle length in the limb with a history of ACL injury may increase its susceptibility to muscle damage during powerful eccentric contractions that occur during periods of high speed running. This increased susceptibility to muscle damage may then contribute to the increased HSI risk in individuals with a history of ACL injury.

Paragraph 25

Although speculative from the current data, changes in muscle activation throughout the entire knee range of motion may contribute to variations in muscle architecture in individuals with a history of ACL injury. Certainly individuals with a previous hamstring strain injury display less BF_{lh} activation at long muscle lengths, which hypothetically might be mediated by the pain associated with the initial injury (11, 27, 34). Investigations into experimentally induced pain have shown alterations in muscle activation, mechanical behaviour and motor unit discharge rates in an apparent effort to reduce stress (force per unit area) and protect the painful structures from further discomfort(11, 12, 20). Therefore the pain associated with an ACL injury, as well as the surgical reconstruction, may alter knee flexor muscle activation so as to protect the knee from further discomfort. If these alterations in muscle activation are accentuated at long knee flexor muscle lengths, this may then result in architectural maladaptations of the knee flexors. However it is possible that reductions in fascicle length can occur despite compensatory increases in BFlh muscle volume in the ACL injured limb (33), as changes in muscle architecture can occur independent of muscle size (23). What is still to be determined is why and/or how ACL reconstruction using the ipsilateral ST might influence BF_{lh} architecture. It is possible that reductions in activation and eccentric strength may have contributed to the architectural alterations within the BFlh, however other factors may influence these changes. Without architectural data of the other knee flexor muscles (see limitations section), it is impossible to know if these architectural deficits are evident in all the hamstring muscles in the previous ACL injured limb. It is unlikely, however, that there is a unique stimulus to the BF_{lh} compared to the medial hamstrings. Future research should investigate if the architectural differences, found in the BF_{lh}, exist in the neighbouring knee flexors.

Paragraph 26

In this study, individuals with a unilateral ACL injury reconstructed from the ipsilateral ST displayed a significantly lower amount of eccentric strength during the Nordic hamstring exercise in the previously ACL injured limb when compared to the contralateral uninjured limb (15.9%; d = 0.51), despite smaller differences in MVIC strength (5.1%; d = 0.31). Similar between limb differences in eccentric knee flexor strength (16.9%) are evident in individuals with a unilateral ACL injury when assessed via isokinetic dynamometry more than 20 years following the injury (36). With respect to the link between prior ACL injury and HSI, elite Australian footballers who subsequently went on to sustain a HSI were ~14% weaker compared to those that remained injury free when assessed prospectively(24). This is a similar magnitude of weakness seen in the previously ACL reconstructed limb compared to the contralateral uninjured limb in the current study. Given that approximately 60% of HSIs occur during high speed running, these low levels of eccentric strength may suggest a reduced ability to decelerate the lower limb during the terminal swing phase of high speed running(24, 26). This coupled with the previously hypothesized increased susceptibility for muscle damage due to shorter muscle fascicles (2, 9), may increase the risk of a future strain injury of the BF_{lh} in individuals with a previous ACL injury during high speed running or other repetitive eccentric contractions. Additionally, the lower levels of eccentric strength, without any differences in MVIC, in the previously ACL injured limb may be due to a maladaptive tension limiting mechanism (9). As the stresses and strains on the musculoskeletal structures are greater during eccentric contractions compared to isometric efforts (6), it is possible that the lower levels of force during the Nordic hamstring exercise may act to reduce tissue loading in the ACL injured limb.

Paragraph 27

We acknowledge that there are limitations associated with the study. Firstly, the investigation of the muscle architectural characteristics only occurred in the BF_{lh} and therefore it is unknown as to what extent the other knee flexors may also be altered. Indeed previous research suggests that compensatory adaptations may occur where inter-muscular coordination is altered to accommodate the injured muscle (32). We have attempted imaging of the ST and initial data did not display acceptable reproducibility. Previous studies have also reported lower reliability when assessing ST when compared to BF_{lh} with intra-class correlations 0.77 and 0.91 respectively (14). Additionally, as the BF_{lh} is the most commonly injured hamstring muscle (18, 24), we believe that the findings reported in BF_{lh} architectural differences between limbs with and without ACL reconstruction are of importance. Future work should examine if these architectural differences are present in the other knee flexors, particularly the harvested ST. Secondly the retrospective nature of the study limits the determination of whether the differences in muscle architecture and eccentric strength existed prior to the ACL injury and reconstruction or were the result of the incident. Prospective investigations are required to determine any existence of a causal relationship and should be the focus of future research. Finally, the current study only included athletes with an ACL injury which was reconstructed with a graft from the ipsilateral ST. Future research should aim to investigate the architectural variations in athletes with a non-ST graft.

Paragraph 28

In conclusion, the current study provided evidence that BF_{lh} fascicle length, pennation angle and eccentric knee flexor strength during the Nordic hamstring exercise, in individuals with a unilateral ACL injury which was reconstructed from the ipsilateral ST, is significantly different to limbs without a history of ACL injury. Despite the retrospective nature of these findings, they provide significant insight into the architectural and eccentric strength asymmetries of the BF_{lh} which exist in those who have a history of ACL injury. These differences should be considered when attempting to limit the risk of future HSI in those with a history of ACL injury. Much work is still required to determine if hamstring muscle architecture and eccentric knee flexor strength play a role in the aetiology of an ACL injury.

ACKNOWLEDGMENTS

Paragraph 29

Dr. Anthony Shield and Dr. David Opar are listed as co-inventors on an international patent application filed for the experimental device (PCT/AU2012/001041.2012). Results of this study do not constitute endorsement of the American College of Sports Medicine

Disclosure of funding:

This study was partially funded by a Faculty of Health Research Grant from the Australian Catholic University.

REFERENCES

1. Blazevich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *J Anat*. 2006 Sep;209(3):289-310.

2. Brockett CL, Morgan DL, Proske U. Predicting hamstring strain injury in elite athletes. *Med Sci Sports Exerc*. 2004 Mar;36(3):379-87.

3. Butterfield TA, Herzog W. The magnitude of muscle strain does not influence serial sarcomere number adaptations following eccentric exercise. *Pflugers Archiv*. 2006 Feb;451(5):688-700.

4. Cohen D. *Statistical power analysis for the behavioral sciences*. Hillsdale (NJ): Erlbaum; 1988. 77-83.

5. Dallalana RJ, Brooks JH, Kemp SP, Williams AM. The epidemiology of knee injuries in English professional rugby union. *Am J Sports Med*. 2007 May;35(5):818-30.

6. Enoka RM. Eccentric contractions require unique activation strategies by the nervous system. *J Appl Physiol (1985)*. 1996 Dec;81(6):2339-46.

7. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007 May;39(2):175-91.

8. Frank CB, Jackson DW. The science of reconstruction of the anterior cruciate ligament. *J Bone Joint Surg Am*. 1997 Oct;79(10):1556-76.

9. Fyfe JJ, Opar DA, Williams MD, Shield AJ. The role of neuromuscular inhibition in hamstring strain injury recurrence. *J Electromyogr Kinesiol*. 2013 Jun;23(3):523-30.

10. Heiderscheit BC, Sherry MA, Silder A, Chumanov ES, Thelen DG. Hamstring strain injuries: recommendations for diagnosis, rehabilitation, and injury prevention. *J Orthop Sports Phys Ther*. 2010 Feb;40(2):67-81.

11. Hodges PW, Tucker K. Moving differently in pain: a new theory to explain the adaptation to pain. *Pain*. 2011 Mar;152(3 Suppl):S90-8.

12. Hug F, Hodges PW, van den Hoorn W, Tucker K. Between-muscle differences in the adaptation to experimental pain. *J Appl Physiol (1985)*. 2014 Nov 15;117(10):1132-40.

13. Jonhagen S, Nemeth G, Eriksson E. Hamstring injuries in sprinters. The role of concentric and eccentric hamstring muscle strength and flexibility. *Am J Sports Med*. 1994 Mar-Apr;22(2):262-6.

14. Kellis E, Galanis N, Natsis K, Kapetanos G. Validity of architectural properties of the hamstring muscles: correlation of ultrasound findings with cadaveric dissection. *J Biomech*. 2009 Nov 13;42(15):2549-54.

15. Klimstra M, Dowling J, Durkin JL, MacDonald M. The effect of ultrasound probe orientation on muscle architecture measurement. *J Electromyogr Kinesiol*. 2007 Aug;17(4):504-14.

16. Knezevic OM, Mirkov DM, Kadija M, Nedeljkovic A, Jaric S. Asymmetries in explosive strength following anterior cruciate ligament reconstruction. *The Knee*. 2014 Jul;21(6):1039-45.

17. Konishi Y, Kinugasa R, Oda T, Tsukazaki S, Fukubayashi T. Relationship between muscle volume and muscle torque of the hamstrings after anterior cruciate ligament lesion. *Knee Surg Sports Traumatol Arthrosc*. 2012 Nov;20(11):2270-4.

18. Koulouris G, Connell DA, Brukner P, Schneider-Kolsky M. Magnetic resonance imaging parameters for assessing risk of recurrent hamstring injuries in elite athletes. *Am J Sports Med*. 2007 Sep;35(9):1500-6.

19. Kramer J, Nusca D, Fowler P, Webster-Bogaert S. Knee flexor and extensor strength during concentric and eccentric muscle actions after anterior cruciate ligament reconstruction

using the semitendinosus tendon and ligament augmentation device. *Am J Sports Med*. 1993 Mar-Apr;21(2):285-91.

20. Lund JP, Donga R, Widmer CG, Stohler CS. The pain-adaptation model: a discussion of the relationship between chronic musculoskeletal pain and motor activity. *Can J Physiol Pharmacol*. 1991 May;69(5):683-94.

21. Makihara Y, Nishino A, Fukubayashi T, Kanamori A. Decrease of knee flexion torque in patients with ACL reconstruction: combined analysis of the architecture and function of the knee flexor muscles. *Knee Surg Sports Traumatol Arthrosc.* 2006 Apr;14(4):310-7.

22. Morgan DL. New insights into the behavior of muscle during active lengthening. *Biophys J*. 1990;57(2):209-21.

23. Noorkoiv M, Nosaka K, Blazevich AJ. Neuromuscular adaptations associated with knee joint angle-specific force change. *Med Sci Sports Exerc*. 2014 Aug;46(8):1525-37.

24. Opar D, Williams M, Timmins R, Hickey J, Duhig S, Shield A. Eccentric hamstring strength and hamstring injury risk in Australian Footballers. *Med Sci Sports Exerc*. 2015;47(4):857-65.

25. Opar DA, Piatkowski T, Williams MD, Shield AJ. A novel device using the nordic hamstring exercise to assess eccentric knee flexor strength: a reliability and retrospective injury study. *J Orthop Sports Phys Ther*. 2013 Sep;43(9):636-40.

26. Opar DA, Williams MD, Shield AJ. Hamstring strain injuries: factors that lead to injury and re-injury. *Sports Med*. 2012 Mar 1;42(3):209-26.

27. Opar DA, Williams MD, Timmins RG, Dear NM, Shield AJ. Knee flexor strength and bicep femoris electromyographical activity is lower in previously strained hamstrings. *J Electromyogr Kinesiol*. 2013 Jun;23(3):696-703.

28. Opar DA, Williams MD, Timmins RG, Dear NM, Shield AJ. Rate of torque and electromyographic development during anticipated eccentric contraction is lower in previously strained hamstrings. *Am J Sports Med*. 2013 Jan;41(1):116-25.

29. Orchard JW, Seward H, Orchard JJ. Results of 2 decades of injury surveillance and public release of data in the Australian Football League. *Am J Sports Med*. 2013 Apr;41(4):734-41.

30. Prodromos CC, Han Y, Rogowski J, Joyce B, Shi K. A meta-analysis of the incidence of anterior cruciate ligament tears as a function of gender, sport, and a knee injury-reduction regimen. *Arthroscopy*. 2007 Dec;23(12):1320-5 e6.

31. Roig M, O'Brien K, Kirk G, Murray R, McKinnon P, Shadgan B, et al. The effects of eccentric versus concentric resistance training on muscle strength and mass in healthy adults: a systematic review with meta-analysis. *Br J Sports Med*. 2009 Aug;43(8):556-68.

32. Silder A, Heiderscheit BC, Thelen DG, Enright T, Tuite MJ. MR observations of long-term musculotendon remodeling following a hamstring strain injury. *Skeletal Radiol*. 2008 Dec;37(12):1101-9.

33. Snow BJ, Wilcox JJ, Burks RT, Greis PE. Evaluation of muscle size and fatty infiltration with MRI nine to eleven years following hamstring harvest for ACL reconstruction. *J Bone Joint Surg Am*. 2012 Jul 18;94(14):1274-82.

34. Sole G, Milosavljevic S, Nicholson HD, Sullivan SJ. Selective strength loss and decreased muscle activity in hamstring injury. *J Orthop Sports Phys Ther*. 2011 May;41(5):354-63.

35. St Clair Gibson A, Lambert MI, Durandt JJ, Scales N, Noakes TD. Quadriceps and hamstrings peak torque ratio changes in persons with chronic anterior cruciate ligament deficiency. *J Orthop Sports Phys Ther*. 2000 Jul;30(7):418-27.

36. Tengman E, Brax Olofsson L, Stensdotter AK, Nilsson KG, Hager CK. Anterior cruciate ligament injury after more than 20 years. II. Concentric and eccentric knee muscle strength. *Scand J Med Sci Sports*. 2014 Dec;24(6):e501-9.

37. Timmins R, Shield A, Williams M, Lorenzen C, Opar D. Biceps femoris long head architecture: a reliability and retrospective injury study. *Med Sci Sports Exerc*. 2015;47(5):905-13.

38. Verrall GM, Slavotinek JP, Barnes PG, Fon GT, Spriggins AJ. Clinical risk factors for hamstring muscle strain injury: a prospective study with correlation of injury by magnetic resonance imaging. *Br J Sports Med*. 2001 Dec;35(6):435-9.

Figure 1: A two dimensional ultrasound image of the biceps femoris long head. This image of the biceps femoris long head was taken along the longitudinal axis of the posterior thigh. From these images it is possible to determine the superficial and intermediate aponeuroses, muscle thickness, angle of the fascicle in relation to the aponeurosis. Estimates of fascicle length can then be made via trigonometry using muscle thickness and pennation angle.

Figure 2: Architectural characteristics of the BF_{lh} in ACL injured limb and the contralateral uninjured limb in the previously ACL injured group at both contraction intensities. A) fascicle length B) pennation angle C) muscle thickness D) fascicle length relative to muscle thickness. Error bars illustrate the standard deviation. * $p < 0.05$ injured vs uninjured.

Figure 3: Comparisons of between leg asymmetry for the architectural characteristics of the BF_{lh} in the previously ACL injured group (uninjured minus injured) to the absolute between leg differences of the control group at both contraction intensities. A) fascicle length B) pennation angle C) muscle thickness D) fascicle length relative to muscle thickness. Error bars illustrate the standard deviation. * $p < 0.05$ injured vs control.